

## INDUCING COLD TOLERABILITY IN SQUASH (*CUCURBITA PEPO L.*) PLANT BY USING SALICYLIC ACID AND CHELATED CALCIUM APPLICATION

MADY, M. A.

Faculty of Agriculture, Department of Botany, Benha University, Qalyubia, Egypt

### ABSTRACT

Two field experiments were conducted at Experimental Farm Station of Fac. of Agric., Moshtohor, Benha University, Egypt to study the effect of foliar application with 0.0, 100 & 150 ppm of salicylic acid (SA) and 0.0, 1000 & 1500 chelated calcium (Ca) and their combinations on some growth aspects, photosynthetic pigments, minerals, endogenous phytohormones, some antioxidant enzymes activity, flowering, fruiting and fruit quality of squash cv. Eskandarani during 2011 and 2012 seasons. Experiments were carried out under open field during winter months at low temperature conditions. Plants were sprayed three times at 20, 35 and 50 days after sowing. Results indicated that, application of SA or Ca individually or in combinations significantly increased most vegetative and reproductive growth traits of squash compared with control. These treatments, also, altered the sex ratio to be in favour of female flowers and led to the earliness of fruit production as well as total fruit yield / plant was significantly increased. Besides, the two concentrations of each applied salicylic acid or calcium obviously increased photosynthetic pigments, NPK, Ca, Mg, Fe, total sugars, total free amino acids and crude protein concentrations in leaves of treated plants as compared with those of untreated ones. Also, most treatments increased auxin, gibberellin and cytokinin levels in squash shoots at 55 days after sowing during 2012 season whereas abscisic acid was decreased. Furthermore, the highest early and total fruit yields were obtained with SA 150 ppm + Ca 1000 ppm followed by SA 100 ppm + Ca 1000 ppm, respectively. Moreover, application of SA or Ca individually or in combinations induced reduction in the peroxidase, catalase and superoxide dismutase activities but increased the nitrate reductase activity as compared with those of the untreated plants in squash leaves at 55 days after sowing during 2012 season. In addition, the contents of some chemical composition of minerals and bioconstituents such as carbohydrates, vitamin C, total soluble solids and nitrate reductase activity in squash fruits were also increased but nitrate- nitrogen content was decreased at the same treatments. Hence, it could be recommended that foliar spraying with salicylic acid at 150 ppm and calcium at 1000 ppm could be used to induce cold tolerability as well as increase the final fruit yield and fruit quality of squash plant under open field at low temperature condition.

**KEYWORDS:** Calcium, Salicylic Acid, Low Temperature, Photosynthetic Pigments, Endogenous Phytohormones, Yield, Squash, Antioxidant Enzymes Activity

### INTRODUCTION

Low temperature is detrimental to plant growth and development and thus affects the productivity of important vegetable crops (Varshney *et al.* 2011 and Shi *et al.* 2012). Squash, (*Cucurbita pepo L.*) is one of the important vegetables grown in Egypt. It is cultivated in Egypt all over the year, outdoor in summer and indoor either in greenhouses or in tunnels in winter. Squash is injured when exposed to low temperatures, i.e., below 12 °C (Rab and Saltveit, 1996).

Squash is susceptible to low temperatures throughout its growth cycle (Wien, 1997). Moreover, Lukatkin *et al.* (2012) shows that the exposure of chilling-sensitive plants to low temperatures causes disturbances in all physiological processes as water regime, mineral nutrition, photosynthesis, respiration and metabolism. Inactivation of metabolism, observed at chilling of chilling-sensitive plants is a complex function of both temperature and duration of exposure.

Various mechanisms have been suggested to account for chilling or tolerance in plants. Some of the changes related to low-temperature stress include alterations in gene expression, proteins, lipids, carbohydrate composition, membrane properties, solute leakage, mitochondrial respiration, and photosynthesis (Basra, 2001). Another mechanism involves a biochemical defense against membrane lipid peroxidation incited by increased generation of reactive oxygen species (ROS) such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $OH$ ), and singlet oxygen ( $O_2^*$ ) in response to chilling (Havaux and Davaud, 1994; Terashima *et al.*, 1994 and Miller *et al.*, 2010).

Salicylic acid (SA) is a phenolic compound and recognized as an endogenous regulator in plants after the finding that it is involved in many plant physiological processes (Raskin, 1992 and Pancheva *et al.*, 1996). Exogenous application of SA may influence a range of diverse processes in plants, including stomatal closure, ion uptake and transport (Harper and Balke 1981), membrane permeability (Barkosky and Einhellig 1993), photosynthetic and growth rate (Khan *et al.* 2003). SA was also shown to enhance the chilling tolerance of various species. This enhanced tolerance was accompanied by increased activation of certain antioxidant enzymes, including glutathione reductase, peroxidase and superoxide dismutase (Horvath *et al.* 2007). On the other hand, SA showed synergistic effect with auxin and gibberellins (Datta and Nanda, 1985 and Sanaa *et al.*, 2006). Also, in a number of species SA promoted flowering in combination with other plant growth regulators such as kinetin, indole acetic acid and gibberellins (Singh, 1984 and Shehata *et al.*, 2000). Applied SA induced changes in endogenous phytohormones of tomato and other plants (Raskin, 1992 and Waffaa *et al.*, 1996).

Calcium (Ca) is an essential plant macronutrient with key structural and signaling roles. Calcium ions ( $Ca^{2+}$ ) act as: an osmoticum within vacuoles; a stabilizing element of membranes; a strengthening agent in cell walls; and a secondary messenger for a multitude of signals (White and Broadley, 2003; McAinsh and Pittman, 2009; Dodd *et al.*, 2010 and Cacho *et al.*, 2013). Calcium is an important messenger in a low temperature signal transduction pathway. The change in cytosolic calcium levels is a necessary first step in a temperature sensing mechanism, which enables the plant to withstand future cold stress in a better way. In both Arabidopsis and alfalfa cytoplasmic calcium levels increase rapidly in response to low temperature, largely due to an influx of calcium from extracellular stores (Mahajan and Tuteja 2005). Moreover, calcium is an important second messenger in plant signaling networks (Cacho *et al.* 2013; Huda *et al.* 2013 and Sarwat *et al.* 2013). Many developmental and environmental stimuli induce the increase of cytosolic calcium to trigger different physiological and downstream responses (Zhu *et al.* 2013). The calcium signaling was achieved by crosstalk of calcium sensitivity, localization and expression of calcium sensors and their downstream partners such as several protein kinases, and the interactions with other signaling pathways (Cacho *et al.* 2013; Huda *et al.* 2013 and Sarwat *et al.* 2013). In plants, exogenous applications of calcium conferred enhanced tolerance to cold stress, (Wang *et al.* 2009 and Zhou and Guo 2009) and modulated stress-induced ROS metabolism, growth performance, photosynthetic efficiency, and nitrogen assimilation (Zhu *et al.* 2013). Therefore, the present study aimed to induce cold tolerability in squash plants by using foliar spray of salicylic acid and chelated calcium to improve growth and productivity under open field at low temperature conditions during winter months.

## MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm Station of Fac. Agric., Moshtohor, Benha University, Egypt, during winter seasons of 2011 and 2012 to study the effect of foliar application with salicylic acid (SA) at 0, 100 and 150 ppm and 0.0, 1000 & 1500 chelated calcium (Ca) individually or in combinations on growth, flowering, fruit yield and quality as well as photosynthetic pigments, minerals content, total sugars, total free amino acid, some antioxidant enzymes activity, flowering, fruiting and fruit quality of squash cv Eskandarani during 2011 and 2012 seasons under open field at low temperature condition. Seed of squash (*Cucurbita pepo L.*) cv Eskandarani secured from the Egyptian Agriculture Research Center, Ministry of Agric., A.R.E. Mechanical and chemical analyses of the experimental soil are presented in Table (A). Mechanical and chemical analyses were estimated according to Jackson (1973) and Black *et al* (1965), respectively.

**Table A. Mechanical and Chemical Analyses of the Experimental Soil Mechanical Analysis**

Soil Particles	Unit	Seasons	
		2011	2012
Coarse sand	%	14.12	15.42
Fine sand	%	14.28	14.35
Silt	%	15.50	15.64
Clay	%	56.10	54.59
Textural class		Clay	Clay

### Chemical Analysis

Parameters	Unit	Seasons	
		2011	2012
Organic matter	%	1.93	1.98
Available N	ppm	62.5	63.6
Available P	ppm	7.5	7.7
Available K	ppm	27.8	28.9
CaCO <sub>3</sub>	%	0.54	0.57
Iron	ppm	25.6	26.5
Zinc	ppm	3.38	3.64
Manganese	ppm	13.54	14.74
Copper	ppm	2.33	2.25
Boron	ppm	14.0	14.5
pH		7.94	7.86

### Climatological Data

Maximum and minimum of air temperature monthly were recorded after Shebeen EL- Kanater weather station.

**Table B: Air Temperature at Qalubia from January to June for the Two Seasons of 2011 and 2012\***

Months	Air Temperature °C Seasons			
	2011		2012	
	Maximum	Minimum	Maximum	Minimum
January	16.5	7.6	15.2	7.2
February	19.7	8.5	19.3	8.4
March	21.8	8.7	20.6	9.3
April	24.5	12.8	23.7	11.5
May	34.2	20.5	32.7	19.5
June	36.5	22.0	37.2	23.0

\*Data after Shebeen EL- Kanater weather station.

## Experimental Design

The experiments included 9 treatments i.e., the control (distilled water), SA at 0.0, 100 and 150 ppm and chelated Ca 0.0, 1000 & 1500 ppm. Seeds of each treatment in both seasons 2011 and 2012 were sown at 15<sup>th</sup> of January in open field in rows on one side of ridge 3.5m length and 0.6 width at 0.4m apart per experimental plot of 10.5m<sup>2</sup> area. The experiments were performed as a randomized complete block design in five replicates. All agricultural practices of growing squash plant including equal amounts of fertilizers and water as well as disease and pests control were followed up. Plants were sprayed three times at 20, 35 and 50 days after sowing.

### Each Experiment Included the Following Treatments

- Control 0.0 (distilled water).
- Salicylic acid (SA) at 100 ppm.
- Salicylic acid (SA) at 150 ppm.
- Calcium (Ca) at 1000 ppm.
- Calcium (Ca) at 1500 ppm.
- (SA) at 100 ppm + (Ca) at 1000 ppm.
- (SA) at 100 ppm + (Ca) at 1500 ppm.
- (SA) at 150 ppm + (Ca) at 1000 ppm.
- (SA) at 150 ppm + (Ca) at 1500 ppm.

### Growth Parameters

Fifty five days after sowing samples randomly were taken following measurements and determinations:

**The Shoot System:** Stem length and diameter as well (at the basal part), stem dry weight, number of leaves, leaf area (according to Derieux *et al.*, 1973) and leaves dry weight.

**The Flowering and Fruiting Stage:** Number of male and female flowers were counted at two days intervals all over the season. The sex ratio was calculated as the rate of male/ female flowers. Also, number and weight of early formed fruits (as the early four pickings) and total fruits.

**Photosynthetic Pigments:** Chlorophyll a, b and carotenoids were calorimetrically determined in squash leaves at 55 days after sowing according to the method described by Inskeep and Bloom (1985) and calculated as mg/g fresh weight.

**Chemical Analysis:** Total Nitrogen, phosphorus, potassium, calcium and magnesium, iron, sugars, carbohydrates and total free amino acids (in leaves at 55 days after sowing as well as in the fresh marketable sized picked-fruits) were determined according to the methods described by Horneck and Miller (1998), Sandell (1950), Horneck and Hanson (1998), Jackson (1973), Thomas and Dutcher (1924) and Dubois *et al* (1956) and Rosed (1957), respectively. Also, crude protein was calculated according to A.O.A.C. (1990) using the following equation.

Crude protein = total nitrogen X 6.25. In addition, in fresh fruits, a hand refractometer and the method of A. O. A. C. (1990) were used for the total soluble solids and vitamin C and titratable acidity determinations, respectively.

**Endogenous Phytohormones** were quantitatively determined in squash shoots at 55 days after sowing in the second season using High-Performance Liquid Chromatography (HPLC) according to Koshioka *et al.* (1983) for auxin (IAA), gibberellic acid (GA<sub>3</sub>) and abscisic acid (ABA) while, cytokinins were determined according to Nicander *et al.* (1993).

**Assay of Enzymes Activities:** Assay of catalase, peroxidase and superoxide dismutase and their activities were determined in squash leaves at 55 days after sowing in the second season according to the methods described by Cao *et al.* (2005) and calculated according to the method of Kong *et al.* (1999). Also, nitrate reductase activity and nitrate- nitrogen content were determined in squash leaves at 55 days after sowing as well as in the fresh marketable sized picked-fruits according to the methods described by Jaworsky (1971) and Black *et al.* (1965), respectively.

**Statistical Analysis:** Data of growth, flowering and fruiting were statistically analyzed according to Snedecor and Cochran (1980).

## RESULTS AND DISCUSSIONS

### Growth Characteristics

Data in Table 1 show that the growth characteristics of stem (length, diameter and dry weight), number of leaves, total leaf area and dry weight of leaves per plant were significantly increased by foliar application treatments with salicylic acid (SA) at 100 and 150 ppm and with 1000 & 1500 of chelated calcium (Ca) individually or in combinations compared with those developed in control. The most pronounced effect in this respect was shown with SA 150 ppm + Ca 1000 ppm followed by SA 100 ppm + Ca 1000 ppm at 55 days after sowing during 2011 and 2012 seasons, respectively. On the other words, the temperatures prevailing during the winter plantation, at Qaliobia region, during 2011 and 2012 seasons were not

**Table 1: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Growth Characteristics of Squash Plants at 55 Days after Sowing during 2011and 2012 Seasons**

Treatment		Stem						Leaves					
		Length (cm)		Diameter (cm)		Dry Weight g/ Plant		No. of Leaves/ Plant		Leaf Area / Plant (cm <sup>2</sup> )		Dry Weight g/ Plant	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SA 0.0 ppm	Ca 0.0 ppm	8.25	7.48	1.25	1.28	0.74	0.67	13.20	13.31	857.47	864.62	5.84	5.89
SA 100 ppm		9.24	9.43	1.40	1.42	0.83	0.85	15.45	15.74	1003.63	1022.47	6.83	6.96
SA 150 ppm		10.84	10.77	1.56	1.59	0.94	0.96	16.28	16.33	1057.55	1062.75	7.20	7.24
SA 0.0 ppm	Ca 1000 ppm	9.36	9.52	1.48	1.46	0.88	0.92	16.80	16.82	1091.33	1096.52	7.43	7.47
SA 100 ppm		10.88	10.96	1.73	1.75	1.03	1.08	18.45	18.52	1198.51	1203.16	8.16	8.21
SA 150 ppm		12.75	12.68	1.90	1.94	1.15	1.18	19.80	19.84	12.86.21	1292.70	8.76	8.84
SA 0.0 ppm	Ca 1500 ppm	8.78	8.92	1.39	1.41	0.84	0.86	16.42	16.48	1089.50	1120.15	7.26	7.29
SA 100 ppm		10.12	10.30	1.58	1.62	0.95	0.98	17.76	17.93	1153.69	1164.73	7.86	7.90
SA 150 ppm		11.55	11.47	1.65	1.68	1.08	1.12	18.22	18.30	1173.20	1181.50	7.94	7.98
LSD at 5%		0.31	0.45	0.19	0.22	0.06	0.08	1.22	1.36	54.60	62.35	0.25	0.32

Favorable for squash plants grown on the open field and the applied treatments enabled squash plants to grow under these field conditions. With regard to the stimulatory effect of SA and Ca on different estimated characteristics of squash growth it could be attributed to the effect of this components upon the rising of endogenous phytohormones synthesis specially the growth promoters i.e. auxins, gibberellins and cytokinins (Table 6). Also, Gharib (2007) on basil and marjoram and Fathy *et al.*, (2003) on eggplant they mentioned that foliar application with salicylic acid increased plant height, number of branches and leaves per plant and dry weight as well. In addition, These results are in agreement with Imami *et al.* (2011) who reported that foliar spray of SA increased growth characteristics including fresh and dry weights

of chickpea plants in response to chilling stress tolerance. Also, Abou El-Yazeid (2011) mentioned that foliar application with SA increased number of branches and leaves, total leaf area and dry weight of leaves of sweet pepper plants under low temperatures on the open field conditions. In this respect, Sayyari (2012) in cucumber and Sayyari *et al.* (2013) in watermelon seedling found that application of SA as a seed soaking or foliar application improved vegetative growth of cucumber and watermelon plants under chilling stresses.

### Photosynthetic Pigments

Data in Table 2 clearly indicate that different foliar application treatments with SA and Ca individually or in combinations significantly increased the photosynthetic pigments as chlorophyll a, b and carotenoids in leaves of squash plants at 55 days after sowing during 2011 and 2012 seasons compared with untreated plants. The most pronounced effect in this respect was shown with SA at 150 ppm + Ca at 1000 ppm followed by SA 100 ppm + Ca 1000 ppm, during two seasons of 2011 and 2012 compared with control plants, respectively. Also, This stimulative effect of the combination of SA and Ca might be due to their antioxidant scavenging effect to be protected chloroplasts and prevented chlorophyll degradation by the toxic

**Table 2: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Photosynthetic Pigments (Mg/G F.W) of Squash Leaves at 55 Days after Sowing during 2011 and 2012 Seasons**

Treatment		Chlorophyll (a)		Chlorophyll (b)		Chlorophyll (a+b)		Carotenoids		Chlorophyll a+b / Carotenoids	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SA 0.0 ppm	Ca 0.0 ppm	0.424	0.443	0.376	0.384	0.800	0.827	0.385	0.392	2.078	2.110
SA 100 ppm		0.548	0.554	0.431	0.436	0.979	0.990	0.446	0.448	2.205	2.210
SA 150 ppm		0.564	0.568	0.460	0.465	1.024	1.033	0.450	0.455	2.276	2.270
SA 0.0 ppm	Ca 1000 ppm	0.587	0.586	0.450	0.452	1.037	1.038	0.462	0.466	2.245	2.227
SA 100 ppm		0.618	0.621	0.482	0.486	1.100	1.107	0.489	0.492	2.249	2.250
SA 150 ppm		0.625	0.624	0.494	0.496	1.119	1.120	0.495	0.496	2.261	2.258
SA 0.0 ppm	Ca 1500 ppm	0.558	0.562	0.436	0.439	0.994	1.001	0.442	0.446	2.249	2.244
SA 100 ppm		0.573	0.577	0.472	0.475	1.045	1.052	0.465	0.468	2.247	2.248
SA 150 ppm		0.589	0.593	0.476	0.480	1.065	1.073	0.478	0.479	2.228	2.240
LSD at 5%		0.04	0.06	0.3	0.05	0.13	0.16	0.04	0.05	0.11	0.08

Reactive oxygen radicals (Bowler *et al.*, 1992 and Aono *et al.*, 1993). Moreover, These results are in agreement with those of Khodary (2004) who found that SA treatment increased the chlorophyll and carotenoids content in maize plants. Imami *et al.* (2011) who found that SA foliar and soil applications increased chlorophyll content in chickpea plants under chilling stress. Environmental stresses mainly reduces chlorophyll content and this reduction depend upon the plant's genotype (Colom and Vazzana, 2001). Based on the theory of Schutz and Fangmeir (2001), the reduction of chlorophyll due to low temperature stress is related to high production of reactive oxygen species (ROS) in the cell. These free radicals cause peroxidation, disintegration and reduction of chlorophyll content in plant under chilling stress. Moreover, SA and Ca with scavenging of ROS and improvement chlorophyll content in plants growing under low temperature. Therefore, impact of SA and Ca on chlorophyll may be related to its influence on the antioxidative enzyme activities and hydrogen peroxide metabolism. Also, Abou El-Yazeid (2011) show that foliar application with SA increased photosynthetic pigments as chlorophyll a, b and carotenoids in leaves of sweet pepper plants under low temperatures on the open field condition.

### Some Minerals and Bioconstituents in Squash Leaves

With regard to minerals concentrations in squash leaves data in Table 3 clearly indicate that different foliar

treatments with SA and Ca individually or in combination significantly increased N, P, K, Ca, Mg, and Fe contents in leaves of squash plants at 55 days after sowing during 2011 and 2012 seasons. Also, it could be noticed that the highest increase of any of these elements was reached with SA at 150 ppm + Ca at 1000 ppm treatment.

As for total bioconstituents concentrations data in Table 4 clearly indicate that, sugars (included reducing and non-reducing sugars) and total free amino acids as well as the crude protein contents were positively responded to the different applied treatments in leaves of squash plants at 55 days after sowing during the two seasons

**Table 3: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Some Nutrients Content of Squash Leaves at 55 Days after Sowing during 2011 and 2012 Seasons**

Treatment		N mg/g D.W.		P mg/g D.W.		K mg/g D.W.		Ca mg/g D.W.		Mg mg/g D.W.		Fe ppm	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SA 0.0 ppm	Ca 0.0 ppm	23.40	23.60	3.12	3.18	25.42	23.58	29.58	29.87	6.24	6.44	54.37	59.46
SA 100 ppm		26.35	25.85	3.44	3.56	28.80	26.75	31.45	31.60	7.46	7.75	66.29	67.42
SA 150 ppm		27.50	27.75	3.75	3.82	29.27	30.15	32.70	33.25	7.54	7.60	70.50	70.76
SA 0.0 ppm	Ca 1000 ppm	28.65	28.50	4.53	4.64	32.78	33.48	35.47	35.94	8.46	8.84	73.76	74.24
SA 100 ppm		30.50	30.45	4.75	4.70	36.50	37.35	37.75	38.26	9.59	9.63	77.86	78.47
SA 150 ppm		33.75	33.60	4.84	4.93	38.44	39.20	38.95	39.66	10.26	10.49	84.58	86.40
SA 0.0 ppm	Ca 1500 ppm	27.90	27.95	4.45	4.48	30.14	31.73	34.55	34.70	7.67	7.78	71.35	72.18
SA 100 ppm		28.40	28.70	4.65	4.69	33.26	34.22	36.28	36.50	7.84	7.92	73.50	73.80
SA 150 ppm		29.55	29.80	4.72	4.74	35.70	36.28	37.56	37.76	8.20	8.27	75.36	76.56
LSD at 5%		1.32	1.35	0.20	0.27	2.30	2.12	1.74	1.54	1.04	1.10	5.42	5.65

**Table 4: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Some Biochemical Contents of Squash Leaves at 55 Days after Sowing during 2011and 2012 Seasons**

Treatment	Sugars mg/g F.W						Free Amino Acids mg/g F.W		Crude Protein mg/g D.W		
	Reducing		Non-Reducing		Total						
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	
SA 0.0 ppm	Ca 0.0 ppm	11.36	11.56	7.07	7.13	18.43	18.69	10.13	10.54	146.25	147.50
SA 100 ppm		15.85	16.75	7.32	7.51	23.17	24.26	13.26	13.35	164.69	161.56
SA 150 ppm		18.20	18.53	7.69	7.92	25.89	26.45	13.40	13.48	171.88	173.44
SA 0.0 ppm	Ca 1000 ppm	19.34	19.64	9.20	9.23	28.54	28.87	13.85	14.17	179.06	178.13
SA 100 ppm		20.26	20.72	9.66	9.68	29.92	30.40	14.45	14.60	190.63	190.31
SA 150 ppm		22.34	22.84	9.76	9.95	32.10	32.79	15.64	15.85	210.94	210.00
SA 0.0 ppm	Ca 1500 ppm	18.12	18.43	9.07	9.13	27.19	27.56	13.68	13.70	174.38	174.69
SA 100 ppm		19.35	19.80	9.15	9.36	28.50	29.16	13.74	13.83	177.50	179.38
SA 150 ppm		19.35	19.82	9.43	9.55	28.78	29.37	13.89	14.35	184.69	186.25
LSD at 5%		1.02	1.06	0.64	0.75	1.14	1.17	0.11	0.16	10.28	12.15

Of 2011 and 2012 compared with control. Regarding the enhancement of the applied treatments upon the minerals contents it could be considered as a direct effect of these treatments upon stimulating their absorption through vigorous root system meanwhile shoot growth characteristics (Table 1), such as the length, diameter and dry weight of stem in plants grown up from foliar application treatment were high significantly increased. Besides, significant increases of the leaf area (Table 1) and increment of the photosynthetic pigments (Table 2) with different treatments were reversed into magnitude of dry matter accumulation in different squash organs (Table1) preceded with increasing of photosynthetic efficiency thereby increment of sugars synthesis and amino acids formation (Table 4). Moreover, Increased sugars in the cell are thought to have a number of roles in improving cold tolerance. Sugars are thought to associate with the membrane of the cell by replacing lost water and maintaining membrane fluidity. Also, sugars play a role as energy sources and building blocks for other important cold tolerance processes in the cell McKown (1996).

Additionally, the main function of anti-oxidants such as SA and Ca were protective of cell membranes and their

binding transporter proteins ( $H^+$  - ATP ase – membrane pumps), maintained their structure and function against the toxic and destructive effects reactive oxygen species (ROS) during stress, in turn, more absorption and translocation of minerals (Dickinson *et al.*, 1991). Also, similar results were obtained by Fathy *et al.*, (2000 & 2003). Moreover, different treatments of squash plants production or synthesis of cryoprotectant molecules such as soluble sugars and low-molecular weight nitrogenous compounds free amino acids. These, in conjunction with dehydrin proteins and cold-regulated proteins act to stabilise both membrane phospholipids and proteins, and cytoplasmic proteins and scavenge reactive oxygen species (ROS); other solutes released from the symplast serve to protect the plasma membrane from ice adhesion and subsequent cell disruption (Gusta *et al.* 2004 and Chen and Murata 2008). Also, Certain cytoskeleton components participate in cold sensing by modulating the activity of  $Ca^{2+}$  channels following membrane rigidification (Abdrakhamanova *et al.* 2003). Because of its basic role in separating the internal from the external environment, the plasma membrane has been considered as a site for the perception of temperature change (Vaultier *et al.* 2006).

### Antioxidant Enzymes Activity

Plants posses antioxidant system in the form of enzymes such as peroxidase (PX), catalase (CAT) and superoxide dismutase (SOD) these antioxidant enzymes are reported to be increased under various environmental stress Noctor and Foyer (1998). In this respect, data in Table 5 clearly show that different applied treatments induced reductions in the peroxidase, catalase and superoxide dismutase activities meanwhile, nitrate reductase activity was increased as compared with control plants in leaves of squash at 55 days after sowing in 2012 season.

**Table 5: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Some Enzymes Activity and Nitrate Contents of Squash Leaves At 55 Days after Sowing during 2012 Season**

Treatment		Anti-Oxidant Enzymes Activity			Nitrate Reductase Activity (NRA) µg/g f.w./h	Nitrate – Nitrogen NO <sub>3</sub> %
		Peroxidase µg/g f.w./h	Catalase µg/g f.w./h	Superoxide Dismutase µg/g f.w./h		
SA 0.0 ppm	Ca 0.0 ppm	87.459	95.473	112.676	0.649	2.236
SA 100 ppm		64.346	83.768	104.875	0.854	1.685
SA 150 ppm		60.566	80.427	102.658	0.954	1.483
SA 0.0 ppm	Ca 1000 ppm	48.865	75.874	96.746	1.453	0.863
SA 100 ppm		43.504	72.559	93.564	1.487	0.853
SA 150 ppm		40.347	70.675	90.646	1.960	0.821
SA 0.0 ppm	Ca 1500 ppm	57.645	79.467	98.648	1.234	0.975
SA 100 ppm		54.397	77.454	97.159	1.286	0.963
SA 150 ppm		50.874	74.598	96.876	1.320	0.952

These reductions in determined enzymatic antioxidant activity with different applied treatments might be due to their direct scavenging function against the toxic free radicals and their promotional effects on synthesis of internal protective antioxidants, i.e., total sugars, total free amino acids and carotenoids. Similar results were obtained by Noctor and Foyer (1998) and Miller *et al.*, (2010). Moreover, salicylic acid or chelated calcium as a foliar application treatments inducing cold tolerability on squash plants through both enzymatic and nonenzymatic systems protect tissue from reactive oxygen species ROS, which are generated as a result of environmental stresses such as drought and low temperature. Members of the enzymatic antioxidant defense system include superoxide dismutase ascorbate peroxidase and catalase. There appears to be a relationship between antioxidant enzyme activity and chilling tolerance. In chilling-tolerant cucumber cultivars, superoxide dismutase and ascorbate peroxidase activity levels were higher than that of the chilling-susceptible cultivars (Shen *et al.*, 1999). As reported by Chen *et al.* (1993) in response to biotic stress SA accumulates to high level, binds and inhibits catalase (CAT) activity, thereby leading to an increase in  $H_2O_2$  content, which could then initiate the development of systemic acquired resistance, induce activity of ROS-detoxifying enzymes, and

synthesis of antioxidant metabolites. Kang and Saltveit (2002) reported that SA-induced chilling tolerance in maize and cucumber plants might be associated with an increase in the activity of glutathione reductase and per-oxidase.

### Endogenous Phytohormones

Endogenous phytohormones in squash shoots as affected by foliar application with salicylic acid or chelated calcium are shown in Table 6. According to these results, all promoters (auxins, gibberellins and cytokinins) were improved by using salicylic acid and calcium, yet, abscisic acid was decreased.

**Table 6: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Endogenous Phytohormones of Squash Shoots at 55 Days after Sowing during 2012 Season**

Treatment	Auxins		Gibberellins		Cytokinins		Abscisic Acid	
	µg/g Fresh Weight	%(±) Control						
SA 0.0 ppm	Ca 0.0 ppm	14.856	0.00	24.327	0.00	4.763	0.00	1.276
SA 100 ppm		16.364	+ 10.151	27.276	+ 12.122	5.564	+ 16.817	1.145
SA 150 ppm		17.436	+ 17.367	27.654	+ 13.676	5.833	+ 22.465	1.075
SA 0.0 ppm	Ca 1000 ppm	18.548	+ 24.852	28.354	+ 16.553	6.758	+ 41.885	0.945
SA 100 ppm		19.637	+ 32.182	28.837	+ 18.539	6.967	+ 46.273	0.928
SA 150 ppm		19.805	+ 33.313	29.625	+ 21.778	7.835	+ 64.497	0.907
SA 0.0 ppm	Ca 1500 ppm	18.785	+ 26.447	27.849	+ 14.478	6.324	+ 32.773	0.985
SA 100 ppm		19.436	+ 30.829	28.507	+ 17.183	6.726	+ 41.214	0.967
SA 150 ppm		19.594	+ 31.893	28.658	+ 17.803	6.837	+ 43.544	0.956

Foliar application with salicylic acid at 150 ppm and calcium at 1000 ppm gave the maximum values in auxins and cytokinins while they gave the highest reduction of abscisic acid in shoots of squash at 55 days after sowing in 2012 season. These data, could also be of great influence upon different vegetative and reproductive growth. In addition, increasing cytokinin level on the account of auxin could be in favor of increasing the vegetative growth (Table 1) and improvement of photosynthetic pigments content (Table 2) in squash plants. Moreover, SA showed synergistic effect with auxin and gibberellins (Datta and Nanda, 1985). Applied SA induced changeable in endogenous phytohormones of tomato and other plants (Raskin, 1992 and Waffaa *et al.*, 1996). Also, Abou El-Yazeid (2011) show that foliar application with SA increased auxins, and cytokinins content in shoots of sweet pepper plants under low temperatures on the open field condition.

### REPRODUCTIVE GROWTH

#### Sex Expression and Fruit Yield

As shown in Table 7 different applied treatments tended to affect male and female flower numbers and that was more markedly for winter squash cv. Eskandarani during 2011 and 2012 seasons. High significant reduction in the male flower number or increase in female ones were existed.

**Table 7: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Flowering, Sex Expression and Fruit Yield of Squash Plants during 2011 and 2012 Seasons**

Treatment	No. of Flowers / Plant						No. of Fruits / Plant				Yield			
	Male ♂		Female ♀		Sex Ratio ♂/♀		Early		Total		Early (g)/Plant		Total (kg)/Plant	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SA 0.0 ppm	Ca 0.0 ppm	61.34	63.26	21.50	22.43	2.85	2.82	2.34	2.54	14.54	14.63	122.86	127.76	1.89
SA 100 ppm		55.26	54.78	26.43	25.64	2.09	2.14	4.14	4.18	20.35	20.68	195.36	198.53	2.65
SA 150 ppm		52.32	51.70	27.60	27.78	1.90	1.86	4.28	4.32	21.48	21.62	206.21	209.65	2.79
SA 0.0 ppm	Ca 1000 ppm	46.43	45.65	30.63	30.84	1.51	1.48	5.45	5.48	24.56	24.89	235.78	239.64	3.19
SA 100 ppm		43.86	44.33	32.46	32.12	1.35	1.38	5.64	5.69	25.87	25.93	248.45	253.32	3.36
SA 150 ppm		40.39	41.48	34.63	34.90	1.17	1.19	5.89	5.94	26.67	26.85	256.03	267.74	3.47

SA 0.0 ppm	Ca 1500 ppm	48.26	49.23	28.32	28.76	1.70	1.71	5.30	5.31	22.14	22.47	212.54	218.65	2.88	2.92
SA 100 ppm		46.20	45.52	29.65	29.44	1.56	1.55	5.34	5.36	23.45	23.62	225.12	231.40	3.05	3.08
SA 150 ppm		44.56	44.73	30.84	30.65	1.45	1.46	5.40	5.42	23.70	23.84	232.52	237.43	3.11	3.14
LSD at 5%		2.15	2.27	1.16	1.19	0.24	0.26	1.14	1.15	1.77	1.84	15.43	16.18	0.23	0.26

Also, foliar application with salicylic acid at 150 ppm and calcium at 1000 ppm showed the highest significant increase in the female flowers number, and the lowest significant reduction in the male flowers number. Thereby, by virtue of the reduction of the male flowers and increasing the female ones the male/female ratio was dominantly showed its high significant reduction with different treatments compared with the control plants. Concerning these data, it could be concluded that, under cool conditions, the production of female flowers in Eskandarani squash cultivar is favoured, since, SA + Ca treatments allowed seedlings to achieve vigorous growth and to flowering earlier during the cool period of cultivation more than untreated plants. Moreover, SA + Ca treatments were encouraged the carbohydrate formation (Table, 4). In this respect, Ne Smiths *et al.*, (1994) reported that squash plants under low temperature may inhibit the development of male flowers after differentiation, leading to precocious female flowers. They also demonstrated that planting date tends to affect female flowers number more markedly for some winter squash cultivars than for others. In addition, Wien (1997) concluded that conditions which enhance the building up of carbohydrates tend to favour female flower expression while factors reduce carbohydrate build-up, such as temperatures, also increase the tendency for male flower production in the cucurbit vegetables.

As for the effect of different applied treatments upon early and total fruits number and yield data showed that significant increase of early and total fruits number and yield during the two seasons were obtained. Also, it is clear that the highest values were obtained with SA 150 ppm + Ca 1000 ppm followed by SA 100 ppm + Ca 1000 ppm, respectively. Regarding the earliness of squash fruiting and increasing of early fruit yield percentage it could be attributed to the increasing of assimilate supply (Table. 4) under low temperatures that favour femaleness and hence enhancement of fruit growth rates. In this respect, Hubbard *et al.*, (1989) and Marcelis (1993) reported that the higher assimilate levels and carbohydrates supply resulted in increased number and size of fruit cells.

### Some Minerals and Bioconstituents in Squash Fruits

Data in Table (8) show that in the 2011 and 2012 seasons all applied treatments in the assigned two levels-increased minerals content, crude protein %, and carbohydrates in the marketable sized squash fruits compared with the control. Also, it is clear that the highest values were obtained with SA 150 ppm + Ca 1000 ppm followed by SA 100 ppm + Ca 1000 ppm, respectively. As indicated in Table (7) In this respect, it is well established that, yield production in the annual herbaceous vegetable crops of the cucurbitaceae is affected by two factors, first that influence overall plant productivity, and second those determine the partitioning of assimilates to reproductive tissues.

**Table 8: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Some of Biochemical and Nutrient Contents Squash Fruits during 2011 and 2012 Seasons**

Treatment		N (%)		P (%)		K (%)		Crude Protein (%)		Total Carbohydrates mg/g D.W	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
SA 0.0 ppm	Ca 0.0 ppm	1.32	1.35	0.356	0.362	1.32	1.36	8.25	8.44	342.25	346.16
SA 100 ppm		1.50	1.53	0.379	0.382	1.50	1.54	9.38	9.56	367.47	371.30
SA 150 ppm		1.54	1.57	0.385	0.389	1.56	1.60	9.63	9.81	374.32	377.58
SA 0.0 ppm		1.75	1.77	0.467	0.470	1.78	1.80	10.94	11.06	385.78	389.46

<b>SA 100 ppm</b>	<b>1000 ppm</b>	1.85	1.89	0.479	0.483	1.82	1.84	11.56	11.81	393.50	395.35
<b>SA 150 ppm</b>		1.94	1.96	0.487	0.490	1.86	1.92	12.13	12.25	397.65	399.58
<b>SA 0.0 ppm</b>	<b>Ca 1500 ppm</b>	1.68	1.70	0.438	0.443	1.65	1.67	10.50	10.63	380.45	384.96
<b>SA 100 ppm</b>		1.72	1.74	0.452	0.456	1.69	1.72	10.75	10.88	386.82	388.26
<b>SA 150 ppm</b>		1.76	1.78	0.463	0.466	1.74	1.76	11.00	11.13	390.46	392.12
<b>LSD at 5%</b>		0.14	0.16	0.04	0.05	0.12	0.15	0.35	0.43	8.84	9.59

The priority of productive structures for attracting assimilates has been reported in some cucurbitaceous as well as in other plants (Hopkins, 1995, Hendrix, 1995 and Wien, 1997).

As for the fruit quality, data in Table (9) show that in the two assigned seasons all treatments in the applied two levels-increased vitamin C, total soluble solids (T.S.S.) and titratable acidity in the marketable sized squash fruits compared with the control. Since, the effect of salicylic acid and calcium treatments upon fruit quality of squash plant it could be noticed that this treatment provided conditions make fruits to be more favorable for marketing with good quality. Also, foliar application treatments with SA and Ca individually or in combination significantly increased nitrate reductase activity meanwhile nitrate nitrogen content was reduced in squash fruit under low temperature condition compared with control fruits.

**Table 9: Effect of Salicylic Acid (SA) and Chelated Calcium (Ca) on Nitrate Reductase Activity, Nitrate Content and Quality of Squash Fruits during 2011 and 2012 Seasons**

Treatment		Vitamin C. mg/100 F.W.		Total Soluble Solids (%)		Titratable Acidity (%)		Nitrate Reductase Activity (NRA) µg/g f.w./h		Nitrate – Nitrogen NO <sub>3</sub> %	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
<b>SA 0.0 ppm</b>	<b>Ca 0.0 ppm</b>	16.80	17.35	4.11	4.18	0.424	0.432	0.649	0.653	1.251	1.235
<b>SA 100 ppm</b>		18.65	18.70	4.34	4.39	0.476	0.479	0.845	0.862	1.165	1.149
<b>SA 150 ppm</b>		18.75	18.79	4.41	4.45	0.483	0.486	0.873	0.884	1.124	1.115
<b>SA 0.0 ppm</b>	<b>Ca 1000 ppm</b>	20.87	21.60	4.75	4.77	0.523	0.529	0.967	0.972	0.932	0.929
<b>SA 100 ppm</b>		22.56	22.78	4.79	4.82	0.542	0.548	0.986	0.995	0.864	0.843
<b>SA 150 ppm</b>		22.80	22.92	4.87	4.86	0.563	0.567	1.064	1.104	0.754	0.738
<b>SA 0.0 ppm</b>	<b>Ca 1500 ppm</b>	18.87	19.15	4.49	4.53	0.513	0.516	0.934	0.938	0.984	0.962
<b>SA 100 ppm</b>		19.34	19.45	4.57	4.58	0.518	0.520	0.945	0.950	0.947	0.940
<b>SA 150 ppm</b>		19.69	19.77	4.62	4.67	0.527	0.532	0.955	0.962	0.920	0.911
<b>LSD at 5%</b>		1.20	1.25	0.14	0.17	0.03	0.05	0.07	0.09	0.23	0.28

## CONCLUSIONS AND RECOMMENDATION

In general it could be concluded that foliar application with salicylic acid and chelated calcium exhibited vigorous seedling growth, altered the gender of the formed flowers to be in favour of female ones. Besides, the foliar treatments also caused earliness of fruit production under low temperatures, increased the total fruit yield/plant improved fruit quality and shortened harvest periods (i.e., minimized that time is needed for fruit to reach the marketable size in the other words to maximize the early fruit yield). All of these advantages could be attributed to the increment of the canopy photosynthesis rate and, in turn, the sufficient assimilates supply. Hence, acceptable great fruit yield with a great portion of early fruits with good quality is being achieved.

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